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REDUCTION OF HEXACHLOROETHANE TO TETRACHLOROETHYLENE IN GROUNDWATER

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ABSTRACT

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At the Canadian Forces Base, Borden, hexachloroethane (HCE) that was introduced into an unconfined sand aquifer disappeared rapidly, with a half-life of about 40 days. Laboratory-scale studies, initiated to help assess the fate of HCE, indicated that it is reductively biotransformed to tetrachloroethylene (PCE) both by aerobic cultures of wastewater microflora and by microcosms containing unhomogenized Borden aquifer material. The results also indicate that the agents involved in the aquifer transformation of HCE to PCE are not homogeneously distributed in the aquifer material.

INTRODUCTION

At the Canadian Forces Base Borden in Ontario, Canada, a group of investigators from Stanford University and the University of Waterloo initiated a natural gradient field experiment to evaluate factors affecting the movement and fate of organic chemicals in groundwater (Mackay et al., 1986). A mass of water ($12\,m^3$) was injected that contained chloride and bromide as inorganic tracers, and five organic solutes: carbon tetrachloride, bromoform, tetrachloroethylene, o-dichlorobenzene, and hexachloroethane. The resulting solute plumes were monitored for about two years using a dense network of multilevel sampling wells. Hexachloroethane (HCE) disappeared much more rapidly than any of the other solutes, yielding a half-life of about 40 days (Roberts et al., 1986).

HCE is an EPA-designated priority pollutant used primarily as a volcanization accelerator and solvent in the rubber manufacturing and explosives industries, respectively. It has a solubility of $50\,mg\,l^{-1}$ (Horvath, 1982), a log octanol-water partition coefficient of 3.64 (Curtis, 1984), and is classified as

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moderately toxic, having a threshold limit value (TLV) of 1 ppm based on liver damage (Sax, 1975). Tabak et al. (1981) reported rapid and complete microbial transformation of HCE under aerobic conditions, but transformation products were not identified and volatilization losses were not documented. The two-electron reduction of HCE to tetrachloroethylene (also known as perchloroethylene or PCE) has been reported in studies with iron porphyrin and cytochrome p-450 (Wade and Castro, 1973; Mansuy and Fontecave, 1982, Nastainczyk and Ulrich, 1982). To date, however, no evidence of a microbially mediated reduction has been presented. Laboratory studies reported in the following were instigated to help in evaluating the potential for microbial transformations of HCE, and its likely fate in the Borden aquifer.

BORDEN AQUIFER FIELD EXPERIMENT

The field study was conducted in an uncontaminated portion of an unconfined glaciofluvial sand aquifer with a water table about 1 m below the ground surface. The injected solute-contaminated water mass was 3—4 m in depth and resided 1—2 m below the water table (Mackay et al., 1986). The solutes moved horizontally with the natural groundwater gradient. Dissolved oxygen (DO) profiles taken near the point of injection indicated the native groundwater was aerobic with DO concentrations ranging from about 1 mg l^{-1} at elevations near the center of the injected mass up to 6 mg l^{-1} just above and below the mass. Reduced constituents in the injected water were sufficiently low in concentration so that they had little effect in reducing DO. Results of additional studies indicated that the native groundwater was anoxic at some locations downgradient from the point of injection. However, where HCE disappearance was observed, aerobic conditions appear to have prevailed. Preliminary estimates of microbial concentration by acridine orange direct counts (Wilson et al., 1983) gave values on the order of 10^6 organisms per gram of aquifer material. As yet, no estimates of the relative concentration of aerobic/anaerobic populations have been attempted.

LABORATORY EXPERIMENTAL METHODS

Two studies were conducted at Stanford. Firstly, an investigation was conducted using a standard seeded BOD technique to evaluate the microbial transformation of HCE. Secondly, a core of Borden aquifer material was obtained for use in laboratory microcosm studies of HCE transformation.

In the first or BOD microcosm study, BOD dilution water was prepared according to Standard Methods (APHA, 1980). It was inoculated with settled municipal wastewater seed (1 ml l^{-1}) from the Palo Alto Wastewater Treatment Plant (Palo Alto, Calif.) and aerated to dissolved oxygen (DO)

saturation (9—10 mg DO l^{-1}). Several 120 ml serum bottles were filled with media without headspace. Half of these bottles were spiked with 10 mg l^{-1} mercuric chloride to serve as biologically inhibited controls. All bottles were spiked with HCE in methanol to give an initial concentration of 6.5 μg HCE l^{-1} and 2.5 μl methanol l^{-1}, sealed using teflon septa, and stored upside-down in the dark at 20°C. The added methanol, if oxidized to carbon dioxide and water, would consume no more than 3 mg DO l^{-1}, thus ensuring aerobic conditions throughout the experiment. Following completion of the study, measurements made by the modified Winkler method (APHA, 1980) confirmed the presence of high levels of DO (8.3 mg l^{-1}).

Throughout the study period, individual serum bottle contents were extracted with 2 ml pentane, thus sacrificing the sample. Extracts were analyzed on a Hewlett-Packard gas chromatograph, equipped with a linearized electron capture detector (GC/ECD) at an oven temperature of 120°C. Triplicate samples were analyzed on days 0, 10, and 20, and duplicate samples on days 30, 40, and 60. Argon-methane was the carrier gas and the column (2 m) was packed with UCON HB 2100. Standard solutions of HCE (Chem Service, West Chester, Pa) and PCE (J.T. Baker Chemical Co., Phillipsburg, N.J.) were prepared in methanol.

In the second or Borden microcosm study, Borden aquifer material rather than wastewater was the source of microbial activity. Cores taken from the Borden aquifer in the 1.5—3 m depth interval were shipped to Stanford and stored at 0°C. They were subsequently extruded and pared under sterile conditions, using methods similar to those outlined by Wilson et al. (1983). In this study, however, core material was extruded directly into sterile Whirl-Pac bags. A small hole was cut in the bottom of each bag, and 18 g (wet weight) of aquifer material was forced out through the hole into each sterile 22 ml serum bottle. The bottles were then filled with groundwater from the Borden site, and spiked with an HCE (J.T. Baker Chemical Co., Phillipsburg, N.J.) in methanol solution to give an initial HCE concentration of approximately 50 μg l^{-1} (210 nmoles l^{-1}). Bottles were sealed without headspace with a teflon septum and aluminum cap, vigorously shaken, and stored upside-down in the dark at 20°C.

The groundwater used in this experiment had been previously acidified to pH 5—6 during transport by plane and storage for two months at 5°C to retard microbial activity. Prior to its use, it was neutralized to pH 7 with NaOH, aerated, and cold sterilized by passage through a sterile 0.45 μm filter. Some pH variation may have occurred during the course of the experiment because of the reduced buffering caused by acidification. Two sets of biologically inhibited controls were also prepared: one set was spiked with mercuric chloride (10 mg l^{-1}), and the other was autoclaved. In the test samples, added methanol, if oxidized to carbon dioxide and water, would exert an oxygen demand of 8 mg l^{-1}. Additional oxygen demand may have been exerted by organics in the aquifer material or in the groundwater. Consequently, aerobic conditions were not guaranteed, and anaerobic zones

may have developed within the microcosms. All microcosms were stored in the dark at 20°C.

Samples were analyzed ten days following the inoculation to permit the system to equilibrate with respect to HCE sorption. Subsequent samples were analyzed on days, 17, 24, 38, 52, and 66. Water was withdrawn from the serum bottles using a long-needle syringe, and transferred to 10 ml vials for pentane extraction. As in the first study, samples were analyzed by GC. Testing revealed the presence of a background PCE in the spike solution, which was traced to the HCE crystals used. This complicated data interpretation.

RESULTS

Chemical analyses of the BOD microcosms revealed the formation of PCE as HCE disappeared. Figure 1 shows the increase in PCE as compared to the relatively constant HCE and PCE in the mercuric-chloride-treated controls. Assuming that 1 mole of HCE is converted to 1 mole PCE, a mass balance can be performed. Results of such calculations are shown in Table 1. Here, the measured HCE in the controls is compared with the sum of sample HCE plus PCE (corrected for any PCE detected in the controls).

Results from the Borden microcosm study are summarized in Table 2. Background PCE is estimated to be approximately 5% of the HCE concentration, but it was variable due to differences in sorption among samples. However, no transformation was observed in spiked groundwater blanks in the absence of aquifer material (data not shown).

The aqueous concentrations of HCE plus PCE were below the added concentration of 210 nmoles l^{-1} in all samples, which would be expected because of sorption to the aquifer materials. The sum of HCE and PCE appears to have decreased in all samples with time, especially in the unaltered and mercury-treated samples. Since this did not occur in controls without aquifer material, loss from the serum bottles is unlikely. A possible explanation for the decrease is a slow increase in sorption with time. This is

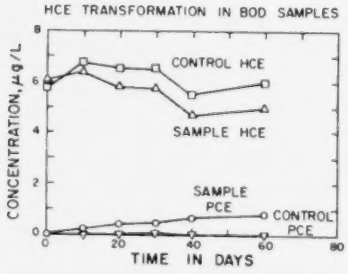

Fig. 1. Hexachloroethane (HCE) transformation to tetrachloroethylene (PCE) in BOD samples.

TABLE 1

Mass balance on HCE and PCE in BOD study. All concentrations in nmoles l^{-1}

Day	Sample			
	PCE[a]	HCE	HCE + PCE[a]	Control HCE
0	0.0	25.6	25.6	24.4
10	1.1	27.1	28.2	28.6
20	2.2	24.5	26.7	27.7
30	2.4	24.3	26.7	27.5
40	3.8	19.8	23.6	23.3
60	4.5	21.0	25.5	25.3

[a]Control PCE = 0.3 ± 0.1 nmoles l^{-1}.

consistent with field observations at Borden (Roberts et al., 1986). The much lower soluble concentration of HCE and PCE in the autoclaved material compared with the other two appears to be the result of increased sorptive capacity of heat treated material as noted by Boyd and King (1984) and on Borden aquifer material by Mackay et al. (this volume).

Transformation of HCE to PCE occurred with unaltered Borden aquifer material just as it did in BOD cultures. However, some transformation also occurred with mercury-treated and autoclaved material. Possible reasons for the latter are given in the discussion and conclusions. In some cases listed in Table 2, significant differences occurred between triplicate samples. For example, with unaltered material for day 17, transformation from HCE to PCE was almost complete in the third sample, while little transformation occurred in the other two. This appears to be a result of the heterogeneity of samples since no effort was made to homogenize the aquifer material before placing it in the serum bottles.

In order to further investigate the variability in HCE transformation noted between samples, aquifer material from the three unaltered material samples analyzed on day 52 was transferred to 25 ml screw-cap vials. The vials were filled with aquifer water, capped, and shaken for one week in the dark on a wrist-action shaker. The light powdery upper fraction that resulted was removed with a Pasteur pipette and analyzed by X-ray diffraction. All three samples contained minerals typical of Borden aquifer material: calcite, quartz, feldspar, and some fine-grained clays. However, the sample in which HCE transformation was nearly complete also contained a distinct, but presently unidentified, component that was not present in the other two samples. Whether this component was associated with the greater extent of transformation is presently unknown, but its presence in only one of the three samples is illustrative of the heterogeneity of the aquifer and probably of the microbial populations.

TABLE 2

Aqueous phase HCE and PCE in Borden microcosm study. All concentrations in nmoles l^{-1}

	Unaltered material			Mercury treated			Autoclaved material		
Day	HCE	PCE[a]	HCE + PCE[a]	HCE	PCE[a]	HCE + PCE[a]	HCE	PCE[a]	HCE + PCE[a]
10	107	3	110	98	3	101	32	27	59
	102	2	104	95	3	98	59	16	75
	109	17	126	99	6	105	63	2	65
17	121	4	125	101	20	121	42	33	75
	119	5	124	100	10	110	59	2	61
	3	128	131	106	12	118	32	15	47
24	74	3	77	69	9	78	39	8	47
	101	5	106	82	8	90	23	26	49
	105	5	110	96	7	103	28	32	60
38	51	6	57	67	8	75	24	14	38
	57	4	61	49	22	81	16	26	42
	44	52	96	57	4	61	33	3	36
52	57	6	63	49	14	63	22	14	36
	6	56	62	58	12	70	25	5	30
	77	14	91	62	16	78	16	32	48
66	57	34	91	52	12	64	28	17	45
	35	58	93	85	11	96	14	27	41
	85	40	125	41	18	59	29	31	60

[a] Background PCE = 4 ± 1 nmoles l^{-1}.

DISCUSSION AND CONCLUSIONS

The study with wastewater seed demonstrated the potential for microbial reduction of HCE to PCE under aerobic conditions. The reaction appears to be a classical example of cometabolism: the product, PCE, is structurally similar to the reactant, HCE; PCE accumulated and did not appear to be further degraded; no acclimation period was required; and the transformation rate appears to have been constant with time. Alexander (1981) proposed that reactions of this class are brought about by enzymes of broad specificity that normally serve a physiological function.

As noted in the introduction, reduction of HCE to PCE has also been reported in at least three other possibly related systems. Wade and Castro (1973) described oxidation of iron(II) deuteroporphyrin IX and reduction of HCE to PCE in pyrrolidone-acetic acid. Nastainczyk and Ulrich (1982) found that extracts of cytochrome p-450, an iron porphyrin-containing cytochrome, also brought about the transformation. Mansuy and Fontecave (1982) developed a chemical model system intended to mimic the behavior of cytochrome p-450. Their system consisted of an aqueous phase, an organic phase (toluene), a reducing agent (sodium ascorbate), iron porphyrin, and a phase transfer agent (tricaprilyl-methyl-ammonium chloride). The latter presumably transports ascorbate into the organic phase where it is oxidized by iron(III) porphyrin. This model system reduced HCE to PCE in the presence or absence of oxygen, but only if the iron porphyrin and the phase transfer agent were both present.

Klecka and Gonsior (1984) found that an anoxic cysteine-ferriprotoporphyrin system resulted in reductive dehalogenation of chloroalkanes with three or more chlorine substituents on the same carbon atoms (1,1,1-trichloroethane, carbon tetrachloride, and chloroform). Other reactions were also promoted by the model system of Mansuy and Fontecave (1982), including the reductive dehalogenation of DDT to DDD. Zoro et al. (1974) used a similar model system to study the transformation of DDT. Their system required the presence of a reducing agent ($FeSO_4$, dithionate, or anaerobic sewage sludge), iron porphyrin (haematin), and a water soluble organic compound (ethanol or Tween 80). The organic phase prevented precipitation of iron porphyrin. The apparent function of components common to both of these model systems are shown in Fig. 2.

In bacteria, cytochromes are reservoirs of iron porphyrins, and pyridine and flavin nucleotides are potential sources of electrons. Thus, one possible explanation for the observed transformation with wastewater bacteria under aerobic conditions is the oxidation of reduced iron porphyrin by HCE. The inhibition caused by mercuric chloride may be due to its action in blocking porphyrin synthesis (Webb, 1966), or to its ability to "outcompete" HCE for the available electrons. Mercuric chloride is known to inhibit electron transport reactions (Webb, 1966).

Interpretation of results for the second or Borden microcosm is somewhat

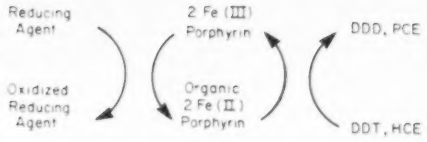

Fig. 2. Key components of iron porphyrin model systems.

difficult due to considerable scatter in the data, the background PCE, and the non-uniform sorption among samples. However, a few general trends emerge:

(1) HCE was converted to PCE in some samples and not in others, suggesting that the active agents were not distributed uniformly throughout the aquifer material (the aquifer material was not homogenized before introduction into the microcosms).

(2) Mercuric chloride appeared to inhibit transformation of HCE to PCE, but to a lesser extent than in the microcosms seeded with wastewater, this may be due to incomplete inhibition in the aquifer microcosms. Fletcher and Kaufman (1980), for example, used a much higher dosage of mercuric chloride ($1\,g\,l^{-1}$) to inhibit biotransformation of 3-chloroaniline in soil samples.

(3) Autoclaving did not prevent transformation, and may have enhanced it.

(4) Autoclaving decreased the recovery of HCE, probably by enhancing its sorption.

The hypothesis that the HCE transformation is enzymatically mediated is not inconsistent with the observations for the second experiment using aquifer material. Autoclaving causes cell lysis and the discharge of cell enzymes into solution, but not necessarily their inactivation. Zoro et al. (1973) reported that autoclaving enhanced the conversion of DDT to DDD by yeast cell suspensions, presumably by increasing the dissolved concentration of an organic reducing agent.

Finally, it would have been fortuitous if the appearance of PCE in laboratory microcosms paralleled field observations from the Borden site. Unfortunately, unequivocable evidence can not be provided because PCE was one of the contaminants injected into the aquifer. However, analysis of PCE concentration data indicate a statistically significant excess mass of PCE over time (Roberts et al., 1986) that would be consistent with formation of PCE from HCE. PCE is known to persist under aerobic conditions, although it is susceptible to transformation under anaerobic conditions (Bouwer and McCarty, 1983). The processes responsible for the loss of HCE in the Borden aquifer remain to be clearly elucidated, but good clues regarding their nature are now available. Information both on the nature and heterogeneous distribution of the agents that transform HCE in the Borden aquifer is needed.

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